REMARKS/ARGUMENTS

With entry of the instant amendment, claims 1 and 22 have been amended and claim 24 has been cancelled. The amendments add no new matter. Support of the amendment to claim 1 can be found, *e.g.,* in claim 24. Claim 22 has been amended to incorporate elements of claim 1 that relate to the lyophilized mixture of thrombin-substrate and $CaCl_2$.

Cancellation of subject matter is without prejudice to subsequent revival for prosecution in a continuation or divisional application.s

Claims 1-8, 10-13, 22, and 23 are pending and under examination.

Applicants thank the Examiner for the telephonic interview on January 23, 2009 in which the experimental data, including experiments 1a, 3, and 4, presented in the Declaration under 37 C.F.R. § 1.132 by Dr. Peter Turecek (referred to in this paper as "the Turecek Declaration") that accompanied Applicants' response filed November 3, 2008 were discussed.

Obviousness rejection

The obviousness rejection is maintained in the final office action mailed November 21, 2008. The Examiner contends that the previous Rule 1.132 Declarations of record in this application did not provide suitable comparative data to show that Applicants' results are truly surprising. In the interests of expediting prosecution, the claims have been amended to relate to a lyophilized mixture that provides a concentration of 1 mM thrombin substrate and 15 mM $CaCl_2$ when reconstituted in an aqueous solution such as water. To the extent that the examiner may contend that the rejection applies to the amended claims, Applicants respectfully traverse.

The Turecek Declaration in fact provides such comparative information, specifically in Experiment 1a when compared to Experiment 3, or Experiment 4. Table 1 provided herein and discussed below is a summary of Experiments 1a, 3, and 4 presented in the Turecek Declaration. The volume of solution used in resuspending the lyophilized mixture for each of these experiments was selected to provide a final concentration of 1 mM fluorescent

thrombin substrate and 15 mM $CaCl_2$, as set forth in the claims. Each resolubilization experiment was performed at least in duplicate (section 7 of the Turecek Declaration).

Table 1: Comparison of Experiments Described in the Turecek Declaration

Experimental Step	**Experiment 1a (Sample 1)**	**Experiment 3 (Sample 3a)**	**Experiment 3 (Sample 3b)**	**Experiment 4 (Sample 4)**
Starting Solution Concentrations	5 mM fl-thrombin 10% DMSO	1 mM fl-thrombin 2% DMSO 15 mM **$CaCl_2$**	1 mM fl-thrombin 2% DMSO 15 mM **$CaCl_2$**	1 mM fl-thrombin 2% DMSO 15 mM **$CaCl_2$** 25 mM HEPES 170 mM NaCl
Lyophilization Step	Yes	Yes	Yes	Yes
Resuspension Volume & Solution Type	5 mL 15 mM **$CaCl_2$** *$CaCl_2$ added post-lyophilization.*	1 mL water	0.2 mL of water + 0.8 mL of 25 mM HEPES/170 mM NaCl	1 mL of water
Final Solution Concentrations*	1 mM fl-thrombin 2% DMSO 15 mM $CaCl_2$	1 mM fl-thrombin 2% DMSO 15 mM $CaCl_2$	1 mM fl-thrombin 2% DMSO 15 mM $CaCl_2$ 20 mM HEPES 136 mM NaCl	1 mM fl-thrombin 2% DMSO 15 mM $CaCl_2$ 25 mM HEPES 170 mM NaCl
Clear or Cloudy Final Solution	***Cloudy;*** *Clear **only*** after vigorous mixing and heating to 37°C	***Clear***; easily dissolved after short vortexing	***Clear*** upon dilution with buffer	***Clear***; dissolved immediately in water

Table 1 Key: fl-thrombin = fluorescently labeled thrombin; Sample and Experiment numbers correspond to those described in the Turecek Declaration. *Final concentrations of DMSO are approximate.

The sample employed in Experiment 1a (Sample 1) had the following concentrations prior to lyophilization: 5 mM fluorescent thrombin substrate and 10% DMSO. The substrate was dissolved and the clear solution was then lyophilized—no $CaCl_2$ was present in the [clear] solution prior to lyophilization and hence, the lyophilized aliquots of this sample. In experiment 1a, an aliquot of this lyophilized mixture was resuspended in 5 mls of 15 mM $CaCl_2$ (to provide a solution where the final volume of substrate is 1 mM and the $CaCl_2$ concentration is 15 mM). However, the substrate was barely soluble (see, section 9 of the Turecek Declaration, as well as Table 1, column 2).

One can directly contrast Experiment 1a with Experiments 3 and 4. For the purposes of comparing concentrations of the fluorogenic thrombin substrate and $CaCl_2$, the discussion focuses on Experiment 3, but the same observations hold true for Experiment 4. In Sample 3, the concentration of fluorogenic thrombin substrate prior to lyophilization was 1 mM. The solution also contained 15 mM $CaCl_2$ and 2% DMSO. The thrombin substrate was dissolved and the clear solution was lyophilized. For Experiment 3a, an aliquot was resuspended in a volume of 1 ml of water (section 11 of the Turecek Declaration and Table 1, column 3). This 1 ml provides a final concentration of 1 mM substrate and 15 mM $CaCl_2$, as set forth in the claims. The mixture was easily dissolved (section 11a, Experiment 3a; Table 1, column 3). Even if re-suspended in 0.2 ml of water followed by adding HEPES buffer (section 11b, Experiment 3b; Table 1, column 4), the initially opalescent mixture was readily dissolved when the volume was brought to 1 ml with HEPES buffer.

This same "readily soluble" property of the lyophilized substrate/$CaCl_2$ mixture was also shown in Experiment 4 (reconstituted with 1 ml of water) where the lyophilized mixture was prepared using a more concentrated HEPES buffer with a higher NaCl concentration (section 12; Table 1, column 5).

Applicants have also provided photographs that are enlarged versions of the pictures submitted with the Turecek Declaration for Experiment 1a, 3a, and 3b showing the solutions following re-suspension of the lyophilized preparation. The vial for 1a shows that the residue was not very soluble whereas the vials for 3a and 3b show clear solutions that readily formed upon dissolving the substrates (see, attached exhibit A and Table 1). Applicants note that these differences in the solutions are clearly visible in the version of the photographs filed with this response. If the images are not clear in the version that the Examiner has access to, Applicants are happy to arrange to provide courtesy copies of these enlarged photographs directly to the Examiner so that they can be viewed without having to be scanned into the USPTO imaging system.

In the office action, the Examiner additionally states that the results are not truly surprising because DMSO may remain following lyophilization and that is why the claimed

mixture is soluble. Applicants do not necessarily agree with this analysis, nor do Applicants concede that this is relevant to the surprising solubility of the lyophilized mixture. However, it is noted that while Sample 1 contains the highest starting concentration of DMSO (10%) it is the least soluble (section 9 compared to section 11; Table 1, column 2 compared to columns 3-4), indicating that the DMSO concentration of the solution prior to lyophilization is not determinative of the increased solubility observed for sample 1a in comparison to samples 3 and 4.

As discussed above and as Dr. Turecek explains in the Turecek Declaration, the ready solubility of the lyophilized fluorescent substrate/$CaCl_2$ mixture in Experiments 3 and 4 was surprising. Applicants have provided comparative evidence that supports that this is an unexpected property of the claimed lyophilized mixture. In view of the foregoing, the invention is unobvious over the cited art. It is respectfully requested that the rejection therefore be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

Jean M. Lockyer
Reg. No. 44,879

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, Eighth Floor
San Francisco, California 94111-3834
Tel: 415-576-0200
Fax: 415-576-0300
Attachments—Exhibit A (3 pages)
JML:jml
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Exhibit A

Experiment 1a

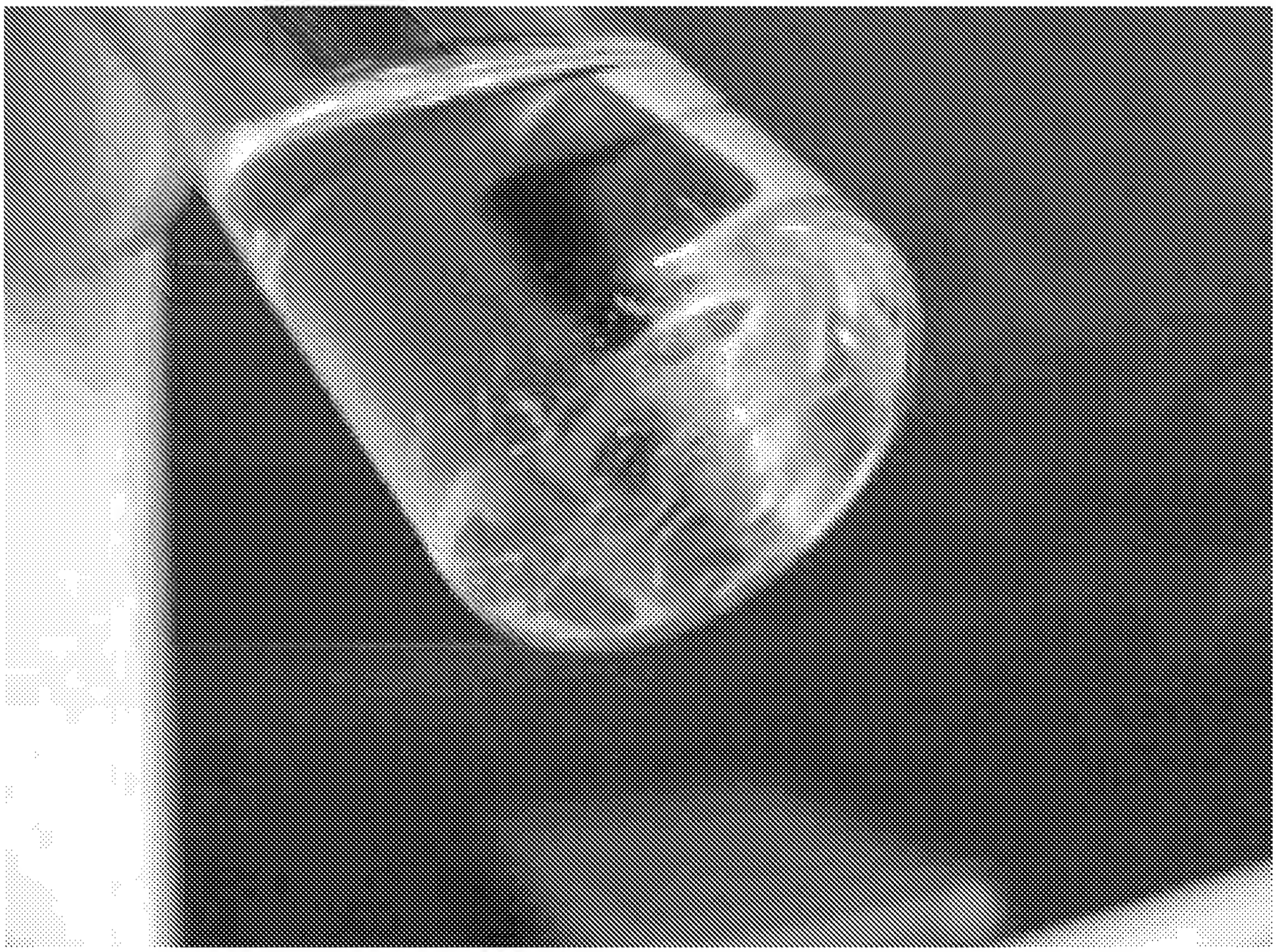

Exhibit A

Experiment 3a

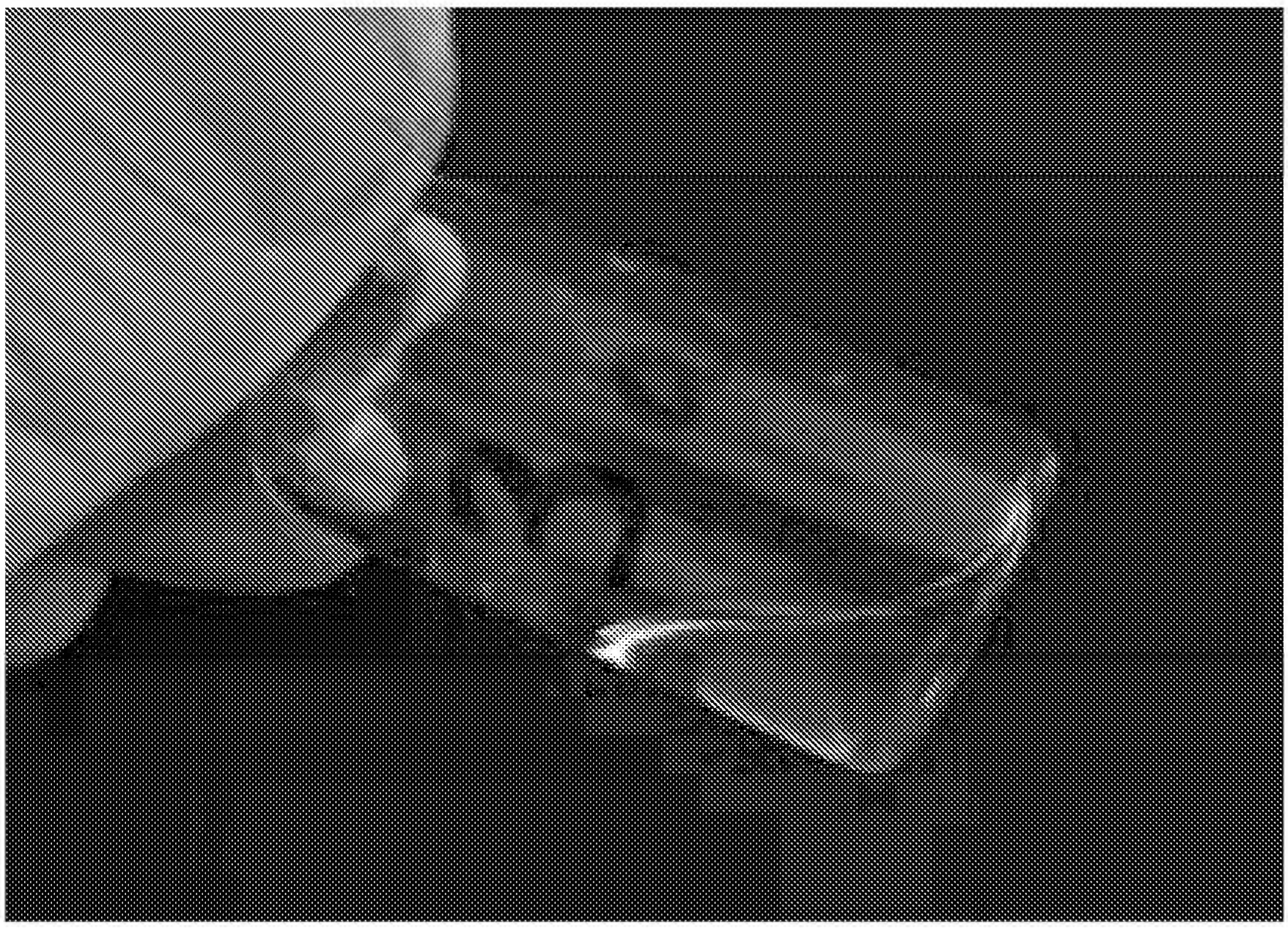

Exhibit A

Experiment 3b